Pilostyles coccoidea (Apodanthaceae), a new species from Western Australia described from morphological and molecular evidence

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Abstract

Thiele, K.R., Wylie, S.J., Maccarone, L., Hollick, P. & McComb, J.A. *Pilostyles coccoidea* (Apodanthaceae), a new species from Western Australia described from morphological and molecular evidence. *Nuytsia* 18: 273–284 (2008). *Pilostyles coccoidea* K.R.Thiele, a new species of holoparasitic flowering plant found on the legume genus *Jacksonia* R.Br. ex Sm., is described and illustrated. The new species is related to *P. collina* Dell and *P. hamiltonii* C.A.Gardner, both also from south-western Western Australia but growing on different hosts. The three species differ in morphological features of flowers and fruits. In addition, analysis of *nad1*, 16S and *matR* gene sequences confirms the distinctness of *P. coccoidea* from *P. hamiltonii*. *Pilostyles coccoidea* appears to be a relatively common species within its restricted range of distribution between Eneabba and the Moore River, north of Perth.

Introduction

Pilostyles Guill. is a genus of *c.* 18 species of holoparasitic flowering plants found in tropical to temperate, arid to semi-arid regions of North and South America, the Middle East and south-western Australia. Previously included in the Rafflesiaceae, recent molecular studies (Barkman *et al.* 2004; Nickrent *et al.* 2004) have indicated that *Pilostyles* and related genera are not closely related to *Rafflesia*, resulting in the segregation of the small family Apodanthaceae Tiegh. ex Takht. for the genera *Pilostyles*, *Apodanthes* Poit. (seven species, tropical South America) and *Berlinianche* Harmsl de Vattimo (two species, tropical East Africa). *Berlinianche* is very similar to *Pilostyles* (Bouman & Meijer 1994) and should probably be included within it (Nickrent 2006).

All species of Apodanthaceae are achlorophyllous, comprising a filamentous mesh-like, endophyte which usually grows isophasically within stems of the host. Flowers are unisexual and develop from primordia formed endogenously within the host cortex just beneath the bark, emerging by rupturing through the stem surface. The flowers comprise one to several series of scale-like bracts and a series of bract-like tepals surrounding a large, central, column-like synandrium (in male flowers) or gynoecium (in female flowers) (Dell *et al.* 1982; Blarer *et al.* 2004).

Pilostyles and *Berlinianche* species parasitise several genera of legumes, while *Apodanthes* is found on a wider range of host families including Salicaceae, Burseraceae and Meliaceae (Blarer *et al.* 2004).

Pilostyles hamiltonii C.A.Gardner was described from Western Australia (Gardner 1948) from material collected on *Daviesia* Sm. at Mundaring. Subsequent collections recorded *Pilostyles* on *Jacksonia* R.Br. ex Sm. in the northern sandplains district between the Moore River and Eneabba, and on several *Gastrolobium*[1] R.Br. species from the Stirling Ranges (Kenneally & Pirkopf 1979) and on Peak Charles and Peak Eleanora in southern south-west Western Australia.

Dell and Burbidge (1981), in a survey of patterns of sexuality of *Pilostyles* flowers growing on different hosts, noted that mixed male and female *Pilostyles* flowers occurred on all stems on *Jacksonia* and *Gastrolobium* hosts, while on *Daviesia* most plants carried only male or female flowers. They concluded from this that the *Pilostyles* individuals on *Jacksonia* and *Gastrolobium* are monoecious, while on *Daviesia* they are dioecious. Occasional *Daviesia* individuals were found in which some stems bore male flowers while other stems bore female flowers, suggesting that the host individuals were probably infected by several *Pilostyles* individuals of differing sexes.

In addition to the difference in sexuality, Dell and Burbidge (1981) noted that *Pilostyles* flowers on different hosts differed in colour, being dark burgundy on *Daviesia*, pink and orange on *Gastrolobium* and orange on *Jacksonia*. They suggested that the three hosts may bear three distinct species of *Pilostyles*; Dell (1983) subsequently erected *P. collina* Dell for the southern, monoecious, pink-and-orange-flowered plants growing on *Gastrolobium*.

Field assessment and morphological and molecular analysis of the northern populations of *Pilostyles* on *Jacksonia* has confirmed that they too comprise a distinct species, which is morphologically and genetically distinct from *P. hamiltonii* on *Daviesia* and morphologically distinct from *P. collina* on *Gastrolobium*. Accordingly, the new species *Pilostyles coccoidea* K.R. Thiele is here described for these populations.

Materials and Methods

Fresh, dried and ethanol-preserved samples of *Pilostyles* flowers were used for morphological comparisons. Fresh or ethanol-preserved fruits and flowers of *Pilostyles* and fresh tip leaves of the hosts *Daviesia angulata* and *Jacksonia floribunda* and of *Lupinus angustifolius* cv. 'Wonga' were used for the DNA extractions (Appendix 1). The host legume samples were collected from plants within infected populations but that appeared to be uninfected with the parasite. These samples, and the *Lupinus* sample, were used to detect possible contamination of parasite DNA with host DNA.

Samples of *Pilostyles coccoidea* were collected from throughout its known range. *Pilostyles hamiltonii* samples were collected from both the northern (Cataby–Badgingarra) and central (Darling Range) parts of its distribution; populations in the southern part of the range near Bunbury were not sampled. Some sampled plants of *P. coccoidea* and *P. hamiltonii* in the Cataby area were less than 100 m apart.

[1] *Oxylobium atropurpureum* Turcz. and *O. linearifolium* C.A.Gardner (= *O. linariifolium* (G.Don) Domin), recorded as hosts for *Pilostyles* in the southern part of its range by e.g. Dell & Burbidge (1981), are now included in *Gastrolobium*, as *G. leakeanum* J.Drumm. and *G. ebracteolatum* G.Chandler & Crisp respectively.

Pilostyles collina appears to be rare and occurs in localized populations in widely scattered locations. Recent extensive searches at known locations (Bluff Knoll, Peak Charles and Hyden) failed to locate plants. Attempts to extract DNA from herbarium samples held at PERTH failed, with the exception of a single specimen from the Hyden area (*A.S. George* 16442) which yielded intact DNA for analysis.

DNA extraction, PCR and sequence analysis. Samples for DNA analysis (100 mg) were frozen in liquid nitrogen, ground to a fine powder and extracted with a DNeasy Plant Miniprep kit (Qiagen). Amplification by polymerase chain reaction (PCR) of DNA sequences comprising the *nad1* (exons 2 and 3 of the mitochondrial NADH dehydrogenase gene), *matR* (mitochondrial maturase R), and 16S (small subunit of the plastid ribosomal RNA) gene regions were done with a 1:1 mixture of *Taq* and *Pfu* DNA polymerases (Promega) using the reaction buffer supplied with the *Pfu* polymerase. *Pfu* polymerase has 3'-5' exonuclease (proof-reading) activity and was used to reduce DNA polymerase-induced nucleotide misincorporations. PCR cycle conditions were as follows: 5 min at 94°C (denaturation) followed by 30 cycles of 94°C for 10 s, 55°C for 30 s, and 72°C for 1 min. Amplifications used the following primers: 16S *8for* (5'–GGAGAGTTCCTGGCTCAG–3') and *1461rev* (5'-GGTGATCCAGCCGCACCTTCCAG-3') (Nickrent *et al.* 1997); *matRfor* (5'-GTTTTCACACCATCGACCGACATCG-3') and *matRrev* (5'-GTTTTCACACCATCGACCGACATCG-3') (Nickrent & Starr 1994); and *nad1b* 5'-GCATTACGATCTGCAGCTCA-3') and *nad1c* (5'-GGAGCTCGATTAGTTTCTGC -3') (Demesure *et al.* 1995).

Both strands of PCR products were sequenced either (i) directly after purification by ethanol precipitation with the primers used in their amplification or (ii) after first cloning into the PCR® Blunt-Topo® (Invitrogen) vector, then using primers *M13F* (5'-TCCCAGTCACGACGTCGT-3') and *M13R* (5'-GGAAACAGCTATGACCATG-3'). Internal primers used to determine the full sequence of 16S were *660f* (5'-TATACTGACGTTGAGGGACG-3') and *990rev* (5'-CCTAACACTTCACTGCACGAACTG-3'), and for *matR matR703for* (5'-AAGTGTTAATAACAATTTAGC-3') and *matR781rev* (5'- CGGTGCTTTACCCGTAGACG -3').

Automated sequencing was performed with an Applied Biosystems Industries (ABI)/Hitachi 3730 DNA Analyzer using BigDye Terminator V3.1 chemistry (ABI).

Sequences were submitted to GenBank and assigned accession numbers (Appendix 1). Additional sequences were obtained from Genbank for *Pilostyles thurberi* (16S, *matR*), *Apodanthes casearieae* (*matR*), *Glycine max* (*nad1*) and *Pisum sativum* (16S, *matR*) for comparative purposes (Appendix 1). Genetic diversity of sequences was deduced from nucleotide sequence alignments using ClustalW (Thompson *et al.* 1994) under default parameters and checked manually. Measures of genetic distance were computed using the Maximum Composite Likelihood model within MEGA4 (Tamura *et al.* 2007). Phylogenies were calculated using four different methods: Neighbor-joining (NJ), Minimum Evolution (ME), Unweighted Pair Group Method with Arithmetic mean (UPGMA), and Maximum Parsimony (MP). With all methods used, evaluation of statistical confidence for nucleotide and amino acid sequence groups was by the bootstrap test (1000 replicates).

Results

Morphologically, *Pilostyles coccoidea* differs from *P. hamiltonii*, in which it has been previously included, in host range, sexuality, flowering position, bract number, flower size and colour, and berry shape, and from *P. collina* in distribution, host range, flower colour and size, and in the number of bracts subtending the flowers (Table 1; Figure 1).

Table 1. Key differences between the three Western Australian species of *Pilostyles*

	P. hamiltonii	*P. coccoidea*	*P. collina*[1]
Distribution	Eneabba to Bunbury	Eneabba to Moore River	Stirling Ranges, Peak Charles, Hyden
Host	*Daviesia*	*Jacksonia*	*Gastrolobium*
Sexuality	Dioecious	Monoecious	Monoecious
Flowering position on host	Always on young (2-year old) stems	Usually on old wood, occasionally also on young stems	On young stems
Flower length	(3.5–)4.0–4.5 mm	(2.0–)2.8–3.9 mm	1.5–2.0 mm
Flower diameter	3.2–3.6 mm	1.8–2.5 mm	2.0–2.4 mm
Flower length/ diameter	1.1–1.3	0.75–1.1	0.8–1.0
Bracts	8–12, in 2 whorls	8–12, in 2 whorls	12–15, in 3 whorls
Bract colour	Dark burgundy	Pale orange-brown	Reddish-orange
Column colour	Pale cream	Dull pinkish-orange	Pink; ovary lemon yellow
Berry	Ovoid-turbinate to almost conical, enclosed and hidden by bracts and perianth to maturity, dull reddish	Depressed-globular, exposed within the erect to spreading bracts and perianth, bright orange-red to scarlet	Depressed-globular, exposed within the bracts (colour unknown)

[1]After Dell (1983)

All four sequence analysis methods (NJ, ME, MP and UPGMA) generated congruent phylograms from the nucleotide sequences. Consensus Neighbor-joining trees for each gene region are shown in Figure 2.

For the three gene regions analysed, there was clear evidence of genetic divergence between *P. coccoidea* and *P. hamiltonii*. No clear phylogeographic structuring was apparent within species, and closely sympatric populations of *P. coccoidea* and *P. hamiltonii* clustered separately. The average genetic distance was low within species (<0.003), and substantially higher between *P. coccoidea* and *P. hamiltonii* (*nad1*=0.070, 16S=0.177, *matR*=0.027 respectively; see Tables 2A–C). Only 16S was successfully sequenced from the DNA extracted from dried herbarium material of the Hyden population of *P. collina*. This sequence grouped closely with samples of *P. hamiltonii* (Figure 2B).

Figure 1. Flowers and fruits of *Pilostyles*. A – C. *P. hamiltonii*; A, B – flowers in situ on host stem (*K.R.Thiele* 3188); C – fruits (*K.R.Thiele* 3245). D – F. *P. coccoidea*; D, E – flowers in situ on host stem (*K.R.Thiele* 3495); F – fruits (*K.R.Thiele* 3242).

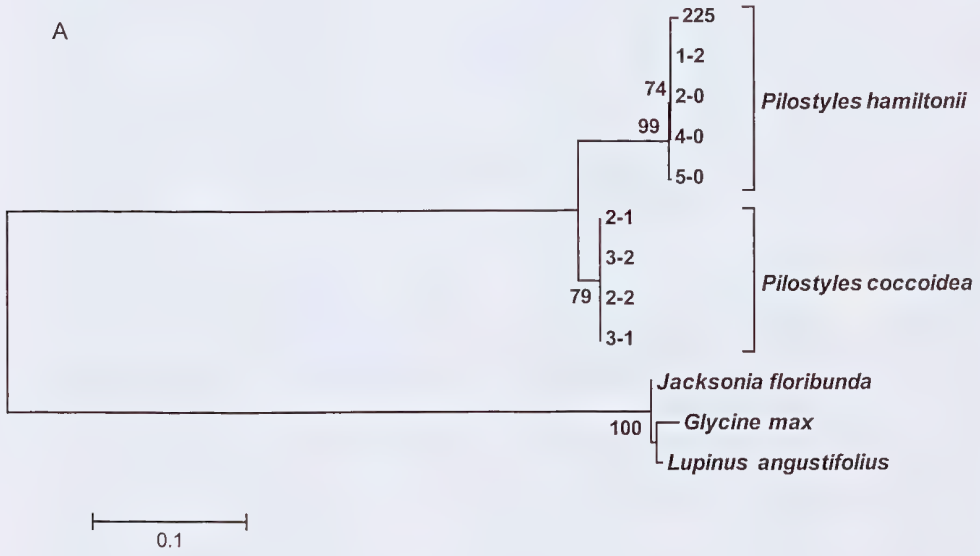
The *nad1* sequences of both *Pilostyles* species were very unusual, differing widely from one another and from those of other plants (Table 2A, Figure 2C). Between them, the two species had eight insertions and deletions (indels) in the *nad1* region sequenced, ranging from 1–72 nucleotides in length. Five indels were present in all five *P. hamiltonii* plants tested and a further three indels were present in all four *P. coccoidea* plants. Compared to other plant-derived nad1 sequences on GenBank, there was high sequence similarity only to short regions of the 5' and 3' termini; overall similarity is estimated to be less than 50%.

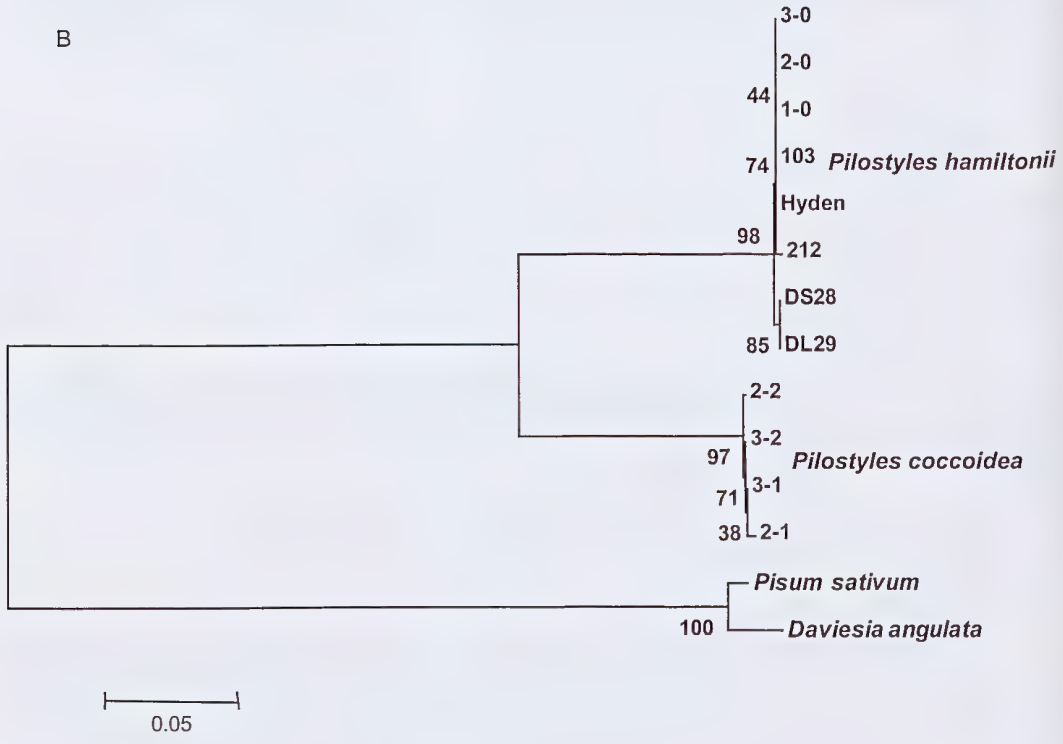
The 16S sequences were also very unusual (Table 2B, Figure 2B). The genetic distance between 16S sequences of the two Australian *Pilostyles* species was high (0.177), and they showed little similarity to those of legumes and, surprisingly, to that of *P. thurberi*.

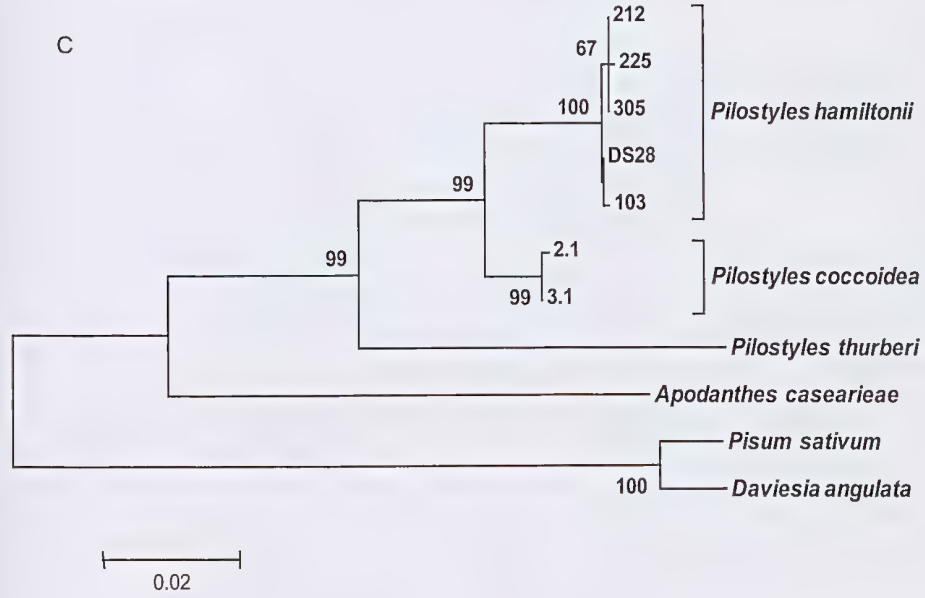
The *matR* sequences of the Australian *Pilostyles* species were more similar to other plants (Table 2C, Figure 2C). As expected, they were closer to *P. thurberi* and *A. caseariae* than to host *matR* sequences.

Host and parasite sequences were clearly differentiated. Genetic distances in the *matR* sequences between the Australian *Pilostyles* and the legume host (where available) were substantial (0.185–0.190). *Pilostyles* 16S and *nad1* sequences showed much lower similarity with host homologues; the genetic distances shown (Figures 2B, 2C; Table 2B, 2C) are approximate because the sequences were so divergent that they were impossible to align with a high degree of confidence.

Figure 2. Neighbour-joining phylograms based on gene sequences for (A) *nad1*; (B) 16S and (C) *matR*; phylograms are drawn to scale, with branch lengths proportional to evolutionary distances. Numbers at the branches are bootstrap values (1000 replications) above 50 percent. The evolutionary distance scale is in the units of the number of nucleotide substitutions per site







Discussion

The low within-species and high between-species divergences of nucleotide sequences confirms the morphological distinctness of *P. coccoidea* and *P. hamiltonii* and supports their classification as distinct taxa.

The existence of eight indels in the *nad1* sequences of *Pilostyles* suggests that the *nad1* gene may be non-functional, and therefore not constrained by natural selection, but this was not proven experimentally. Similarly, the wide divergence of the 16S sequence indicates it may not be functional. On the other hand, the *matR* sequences were similar to homologues from other species and, therefore, may be functional. *MatR* and other mitochondrial genes have been used previously to classify parasitic angiosperms, including *Pilostyles* (Barkman *et al.* 2004; Barkman *et al.* 2007; Nickrent *et al.* 2004).

The relationship of *Pilostyles collina* to the other taxa is not clear. The single specimen that yielded DNA (*A.S. George* 16442) grouped within *P. hamiltonii* on the 16S analysis (Figure 2B). However, *P. collina* is morphologically more similar to *P. coccoidea* than it is to *P. hamiltonii* (Table 1), sharing relatively small flowers compared with *P. hamiltonii* and a berry that is exposed within the short bracts. Cross-contamination with a *P. hamiltonii* sample cannot be ruled out. Until new populations of *P. collina* can be located and fresh material collected, its relationships to the other two species will remain uncertain.

With the recognition of *Pilostyles coccoidea*, each of the three species of *Pilostyles* in Western Australia is considered to be restricted to a single host genus, but to occur on several species within its host genus. In general, host specificity in *Pilostyles* is relatively high, with the entire genus restricted to legume hosts (Nickrent 2006). Some species occur on several host genera (e.g. *P. thurberi* Gray on *Dalea formosa* Torr. and *Psorothamnus emoryi* (Gray) Rydb.). Factors controlling host range in Apodanthaceae are unknown.

Table 2. Mean nucleotide sequence diversity between and within species

A. nad1 sequences

Group	*P. coccoidea*	*P. hamiltonii*	*J. floribunda*	*G. max*	*L. angustifolius*
P. coccoidea	*0.000*[a]	0.070	0.551[b]	0.561[b]	0.555[b]
P. hamiltonii		*0.004*	0.573[b]	0.584[b]	0.578[b]
J. floribunda			-	0.016	0.002
G. max				-	0.018
L. angustifolius					-

B. 16S sequences

Group	*P. coccoidea*	*P. hamiltonii*	*P. thurberi*	*D. angulata*	*P. sativum*
P. coccoidea	*0.002*	0.177	0.601[b]	0.567[b]	0.539[b]
P. hamiltonii		*0.002*	0.608[b]	0.560[b]	0.554[b]
P. thurberi			-	0.701[b]	0.696[b]
D. angulata				-	0.027
P. sativum					-

C. matR sequences

Group	*P. coccoidea*	*P. hamiltonii*	*P. thurberi*	*A. casearieae*	*D. angulata*	*P. sativum*
P. coccoidea	*0.003*	0.027	0.080	0.125	0.185	0.184
P. hamiltonii		*0.001*	0.091	0.135	0.191	0.189
P. thurberi			-	0.152	0.208	0.208
A. casearieae				-	0.195	0.199
D. angulata					-	0.019
P. sativum						-

[a] Genetic distance (number of base substitutions per site as calculated by pairwise analysis).
[b] Figure given for genetic distance is an approximate value because sequences are highly divergent.
Inter-group mean sequence diversity is indicated in plain text. Intra-group mean sequence diversity is indicated in italics.

Early observers (e.g. Smith 1951) expected *Pilostyles hamiltonii* to be very widespread in south-western Western Australia, perhaps extending to the eastern States, on the basis of the wide distribution of the host genus and species. Dell & Burbidge (1981), however, with a more extensive knowledge of its distribution, noted the paradox that the parasite has a substantially more restricted distribution than its hosts. This is true both taxonomically and geographically: both *Daviesia* and *Jacksonia* contain many species not parasitised, and most individual species of host have a wider geographic distribution than the parasite. In particular, *P. hamiltonii* is widespread on *Daviesia* hosts on lateritic soils of the Darling Range but is absent from the same host species on sandy soils of the adjacent Swan Coastal Plain. Similarly, *P. coccoidea* is common on *Jacksonia floribunda* on the Eneabba Sandplains south to the Moore River, but the host species extends considerably further south to near Perth.

Taxonomy

Pilostyles coccoidea K.R.Thiele, *sp. nov.*

A *Pilostyles hamiltonii* floribus parvioribus, bracteis et columna pallida, aurantiaco-brunnea; baccis depresso ovoideis, rubro-aurantiacis, ad maturitatem expositis per bracteas effusas differt.

Typus: Waddi Road, 0.7 km from the Brand Highway, Western Australia, 30° 33' 26" S, 115° 28' 10" E, 7 March 2008, *K.R. Thiele* 3495 (*holo*: PERTH 07692447; *iso*: CANB, K, MEL, MO, NSW).

Pilostyles sp. Northern Sandplains (P. Armstrong *s.n.* PERTH 06590179), Western Australian Herbarium, in *FloraBase*, http://florabase.dec.wa.gov.au [accessed 10 March 2008].

Monoecious, endophytic perennial, the vegetative thallus ramifying within the host tissue. *Flowers* emergent singly from host stems, (2.0–)2.8–3.9 mm long, (1.8–)2.8–3.8 mm diam. (L/W 0.75–1.1) at anthesis, usually closely packed in groups and clusters, often aligned in fissures of bark, globose (although sometimes distorted from close packing). *Bracts* thick, fleshy, broad-based, imbricate, somewhat spreading at anthesis, 8–12 in two whorls of 4–6 each, 1.2–2.6 mm long, 1.0–2.2 mm wide, suborbicular to broadly ovate, the inner whorl longer and narrower than the outer, pale orange-brown darkening and withering at the tips at anthesis. *Perianth segments* 4–5(–8), similar to the bracts but with somewhat attenuate bases. *Disc and column* dull pinkish-orange, epigynous. *Male flowers* with central column (synandrium plus sterile gynoecium) shorter than the perianth, slightly inflated and dome-shaped at the apex, bearing a marginal ring of embedded anther-sacs below a ring of short papillae. *Female flowers* with an inferior to half-inferior, unilocular ovary and a short, thickened, column-like style expanded at the apex with a marginal, papillate stigmatic area and terminal depression; ovules many, on 4 parietal placentas. *Fruit* a depressed-globular, scarlet to orange-red berry surmounted by the prominent, darkened remnants of the stigma, 2.5–3.5(–4) mm diam.; bracts and perianth in fruit erect to spreading, exposing the berry; seeds *c.* 0.4 mm long, ± globular to broadly ellipsoid, corrugate. (Figure 1D–F)

Specimens examined. WESTERN AUSTRALIA: 16 km N of Badgingarra on Brand Highway, 18 May 1995, *P. Armstrong s.n.* (PERTH 06590179); 13.6 km N of Cataby on the Brand Highway, 9 June 1977, *A.H. Burbidge s.n.* (PERTH 01883151); entrance to Allied Eneabba, Brand Highway, Eneabba, 26 Aug. 1976, *B. Dell* 7687a (PERTH); 1 km S of Strathmore Road, on a track 1–2 km W of Brand Highway, 27 Aug. 1977, *B. Dell & A. Burbidge s.n.* (PERTH 03277658); Moore River National Park, 0.6 km at 180 degrees from junction of Red Gully Road and Brand Highway, NW of Gingin, 26 June 1988, *E.A. Griffin* 4775 (PERTH); Brand Highway 22.3 km N of southern roadhouse at Cataby, 20 Mar. 2007, *K.R. Thiele* 3197 (PERTH); Brand Highway 0.6 km N of the turnoff to Cooljarloo Mine, N of Cataby, 12 June 2007, *K.R. Thiele* 3242 (PERTH); Brand Highway at Badgingarra, *c.* 0.1 km N of the turnoff into the town, 12 June 2007, *K.R. Thiele* 3243 (PERTH); Brand Highway at southern turnoff to Iluka Resources Eneabba mine, S of Eneabba, 12 June 2007, *K.R. Thiele* 3246 (PERTH).

Distribution. Pilostyles coccoidea is endemic to the northern wheatbelt region of south-western Western Australia (Figure 3a). All known collections are from the immediate vicinity of the Brand Highway between the Moore River and Eneabba. It almost certainly occurs more widely in the region, but is probably poorly collected. Kenneally and Pirkopf (1979) cite an occurrence at Mt Lesueur, but there is no specimen at PERTH from this locality.

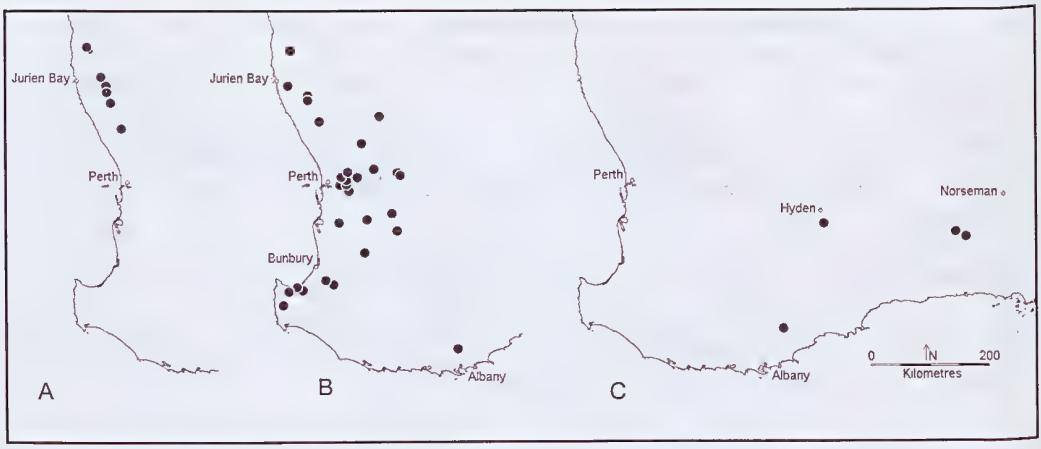


Figure 3. Distribution of *Pilostyles* species in south-west Western Australia. A – *P. coccoidea*; B – *P. hamiltonii*; C – *P. collina*. *Version 6.1 IBRA regions* (Department of the Environment, Water, Heritage and the Arts 2008) are indicated in grey.

Pilostyles coccoidea is sympatric with *P. hamiltonii* (Figure 3B), probably throughout its range, wherever the two hosts co-occur. It is widely allopatric from *P. collina* (Figure 3C).

Habitat. Pilostyles coccoidea has been found on two species of *Jacksonia*, *J. floribunda* Endl. and *J. nutans* Chappill, in low to tall, dense heath vegetation on sandy soil over laterite. Whereas *P. hamiltonii* and *P. collina* consistently flower on young (1–2-year old) stems of their hosts, *P. coccoidea* is usually found on much older wood, sometimes at the base of the plant where its flowers and fruits erupt from fissures in the bark of large, stout stems that are several years old. Flowers are sometimes virtually hidden beneath the papery outer bark layers on *J. floribunda*.

Flowering and fruiting period. Both *P. coccoidea* and *P. hamiltonii* on the northern sandplains flower together in February and March. Fruits persist on the hosts until July or August.

Conservation status. Pilostyles coccoidea appears to be relatively common within its range, and occurs in a number of National Parks and Nature Reserves in the region.

Etymology. The epithet is derived from the Latin *coccus* (a berry) with the termination *–oides* (like, similar), in reference to the remarkable superficial similarity of the fruiting plants to scale insects (Homoptera superfamily Coccoidea), particularly to species such as *Saissetia oleae* and *Eriococcus coriaceus*.

Notes. Pilostyles coccoidea differs most prominently from *P. hamiltonii* in its smaller flowers which are dull orange (dark burgundy in *P. hamiltonii*) and in the berry which is depressed-globular, scarlet and exposed by the spreading bracts (turbinate, dull-coloured and hidden by the erect bracts in *P. hamiltonii*). It differs from *P. collina* in its flower colour (reddish-orange, pink and lemon yellow in *P. collina*; *fide* Dell 1981), northern distribution, and fewer bracts.

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Appendix 1. Gene sequences used in this study.

Species	Sample code	Host species	Locality	Gene[a]	GenBank Accession	Reference
Pilostyles coccoidea	2-1	*J. floribunda*	Badgingarra	16S	EU512422	This study
				matR	EU512427	This study
				nad1		This study
	2-2	*J. floribunda*	Badgingarra	16S	EU512424	This study
				nad1		This study
	3-1	*J. floribunda*	Badgingarra	16S	EU512421	This study
				matR	EU512426	This study
				nad1		This study
	3-2	*J. floribunda*	Eneabba	16S	EU512423	This study
				nad1		This study
Pilostyles hamiltonii	DS28	*D. preissi*	Kalamunda	16S	EF446140	This study
				matR	EU512432	This study
	DL29	*D. decurrens*	Kalamunda	16S	EF446141	This study
	103	*D. angulata*	Kalamunda	*16S*	EF446143	This study
	136	*D. decurrens*	Kalamunda	*nad1*		This study
	212	*D. angulata*	Badgingarra	16S	EF446142	This study
				matR	EU512430	This study
	225	*D. angulata*	Badgingarra	*matR*	EU512424	This study
				nad1		This study
	305 (KRT 3242)	*D. angulata*	Cataby	*matR*	EU512431	This study
	1-0	*Daviesia sp.*	Badgingarra	16S	EU512419	This study
	1-2	*Daviesia sp.*	Eneabba	*nad1*		This study
	2-0	*Daviesia sp.*	Badgingarra	16S	EU512420	This study
				nad1		This study
	3-0	*Daviesia sp.*	Badgingarra	16S	EU512418	This study
	4-0	*Daviesia sp.*	Eneabba	*nad1*		This study
	5-0	*Daviesia sp.*	Eneabba	*nad1*		This study
Pilostyles collina	Hyden (ASG 16442)	*G. spinosum*	Hyden	16S	EU512425	This study
Pilostyles thurberi	2994	*Dalea formosa*	Texas, USA	*matR*	AY739003	Nickrent *et al.*, 2004
			Texas, USA	16S	U67741	Nickrent *et al.*, 1997
Apodanthes caseariae		*Casearia sp.*		*matR*	AY739002	Nickrent *et al.*, 2004
Daviesia angulata	214	-	Badgingarra	*matR*	EU512433	This study
				16S	EF446139	This study
Jacksonia floribunda	304	-	Cataby	*nad1*		This study
Lupinus angustifolius	*cv* Wonga	-	Perth	*nad1*		This study
Glycine max		-	Belgium	*nad1*	AJ428875	unpublished
Pisum sativum		-	N.Y., USA	16S	X51598	Cerutti & Jagendorf, 1991
			MI, USA	*matR*	AY453078	Barkman *et. al.*, 2004

[a]. 16S = plastid 16S ribosomal RNA gene, partial cds; *matR* = mitochondrial maturase R gene, partial cds; *nad1* = mitochondrial NADH dehydrogenase subunit 1 gene, exons 2, and 3 and partial cds.